Heliyon 6 (2020) e04030

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Biomarkers responses of the clam *Anomalocardia flexuosa* in sediment toxicity bioassays using dredged materials from a semi-arid coastal system

Lucas Buruaem Moreira [a,b,*], Silvio Tarou Sasaki [c], Satie Taniguchi [c], Tiago Farias Peres [a], Rubens Cesar Lopes Figueira [c], Marcia Caruso Bícego [c], Rozane Valente Marins [a], Leticia Veras Costa-Lotufo [a], Denis Moledo Souza Abessa [b]

[a] Instituto de Ciências do Mar, Universidade Federal do Ceará, Fortaleza, Brazil
[b] Núcleo de Estudos em Poluição e Ecotoxicologia Aquática, UNESP São Vicente, Brazil
[c] Instituto Oceanográfico, Universidade de São Paulo, São Paulo, Brazil

ARTICLE INFO

Keywords:
Bioaccumulation
Environmental monitoring
Linear alkylbenzenes
Marine pollution
Metals
Polycyclic aromatic hydrocarbons
Sublethal effects
Tropical environments
Toxicity testing
Weight of evidence
Ecological health
Marine biology
Environmental assessment
Environmental hazard
Environmental health
Environmental impact assessment
Environmental pollution
Environmental risk assessment
Environmental toxicology
Toxicology

ABSTRACT

Few test organisms are employed for sediment toxicity assessments in Tropical regions, including Brazil. We assessed the ability of the clam *Anomalocardia flexuosa* to respond to contamination in sediment bioassays using dredging materials of a semi-arid region (Ceará State, NE Brazil), with attention to sublethal responses. Sediments were collected during and after dredging (survey 1 and 2, respectively) and animals exposed in laboratory over 28 days, with responses measured at 7 days. Bioaccumulation of contaminants was determined in whole-body soft tissues as a metric of bioavailability, and biomarkers' changes were monitored in terms of enzymes of phase I and II metabolism, acetylcholinesterase (AChE), and antioxidant responses, lipid peroxidation (LPO) and DNA damage (strand breaks). Clams accumulated aliphatic (AHs) and aromatic hydrocarbons (PAHs), and linear alkylbenzenes (LABs) compared to control conditions (day 0), with increased amounts of As, Cd, Cu, and Zn observed in some samples. The enzyme glutathione S-transferase was enhanced in animals exposed to samples, indicating activation of phase II metabolism. Changes observed in glutathione peroxidase (GPx), glutathione reductase (GR), LPO and strand breaks were related to oxidative stress. AChE enzymatic activity also changed, as an indicator of neurotoxicity caused by sediment exposure. The computed integrated biomarker response index (IBR) ranked sites according to the contamination status and proximity to its sources. Correlations found for biomarkers and bioaccumulation of hydrocarbons indicated the influence of harbor activities, effluent discharges, and urban runoff on the sediment pollution of Mucuripe Bay. Data also showed that SQGs are unable to predict bioaccumulation and subchronic effects. Based on our results we consider that biomarkers responses in *A. flexuosa* are important endpoints to be applied in sediment toxicity bioassays in tropical regions.

1. Introduction

The presence of harbors and their related activities are harmful to coastal ecosystems, from the installation of jetties to the operations of port terminals. Impacts on sediment transport, combined with the discharges of chemical substances from the point and diffuse sources produce a scenario of ecological risk due to dredging materials (NRC, 1997; Moreira et al., 2017). Dredging activities consist of deepening of the seabed by removing sediment particles to change the bathymetry of navigation channels, necessary to the maneuvers of ships. Every year millions of tons of dredged sediments are dumped in the ocean worldwide (OSPAR, 2008; Schipper et al., 2010).

The ecological risks of dredged materials have been performed by following tier-based frameworks including different lines of evidence (LOE) of sediment quality (USEPA, 1991). The most common approach is based on chemical criteria using sediment quality guidelines (SQGs) (Burton, 2002), and as for the ecological effects, sediment toxicity bioassays have been employed using several model organisms from different taxa in laboratory exposures. In these methods, results from classical endpoints (e.g. mortality, development, growth, and reproduction) may be subject to confounding factors, such as ammonia or physical effects of

* Corresponding author.
E-mail address: lburuaem@gmail.com (L.B. Moreira).

https://doi.org/10.1016/j.heliyon.2020.e04030
Received 27 March 2020; Received in revised form 24 April 2020; Accepted 18 May 2020

grain size fractions (Nendza, 2002; Schipper et al., 2010). To deal with that, alternative methods have been recommended as complementary LOEs such as bioaccumulation and biomarkers (Chapman and Hollert, 2006).

The concept of biomarkers consist of changes in status observed at the cellular, biochemical or physiological levels, indicating outcomes of exposure situation and response to contamination, representing thus health indicators of animals exposed for a short-to-medium-term or at environmentally relevant concentrations, which are lower than concentrations commonly used in conventional toxicity testing (Hagger et al., 2006; Hampel et al., 2016). Martín-Díaz et al. (2004) recommend an integrated approach including analyses of phase I (biotransformation) and phase II (conjugation) enzymes, those from the antioxidant system, and also cellular damage such lipid peroxidation and molecular effects like strand breaks of DNA, as relevant endpoints to be included in a proper characterization of dredging materials, in order to avoid misinterpretations caused by confounding factors of laboratory exposures.

Bioaccumulation has also been pointed out as metric of exposure to contaminants either in situ or in laboratory (Moreira et al., 2019a), necessary for a proper risk assessment (Sutter, 2006; USEPA, 1992). This process is marked by the incorporation and storage of chemical substances in organisms via exposure medium (water and sediments), or by the uptake of food containing contaminants at rates faster than detoxification and excretion (DeForest et al., 2007), providing evidence on the bioavailability of chemicals.

The coastal zone of Brazil presents distinct characteristics and the instruments to identify the effects of sediment dredging are regulated by the normative #454 (Brasil, 2012). This resolution is focused on the characterization (particle size analyses and chemical properties) of sediments obtained in the particular area, followed by the comparison of the contamination levels with sediment quality guidelines (SQGs), and adverse effects are measured only in samples presenting contamination levels above threshold values using toxicity bioassays or another complementary ecotoxicological method as a LOE.

One issue is that there are few test organisms for sediment toxicity testing in tropical ecosystems. In Brazil they are included in protocols for acute toxicity in whole-sediment exposure of the amphipod *Tiburonella viscana*, while chronic toxicity of liquid phase is estimated using sea-urchins (*Lytechinus variegatus* and *Echinometra lucunter*) embryo larval development (ABNT, 2006, 2008). Other methods have been developed for the benthic copepods *Nitocra* sp (Lotufo and Abessa, 2002) and *Tisbe biminiensis* (Araújo-Castro et al., 2009), the tanaid *Monokalliapseudes schubarti* (Mottola et al., 2009), and the polychaete *Armandia agilis* (Saes et al., 2018). Those methods are based on classical endpoints that are subject to confounding factors as mentioned before. Hereupon it is important to develop new protocols with different groups such as the mollusks, including other sensitive endpoints as bioaccumulation and biomarkers.

One suitable model is the infaunal clam *Anomalocardia flexuosa* that inhabits the top layers of banks of estuarine deltas, occurring in aggregates from the Caribbean to Brazil. The species is tolerant to hypoxia and salinities ranging from 17 to 38‰, and its optimum temperature range lies between 25 to 36 °C (Silva-Cavalcanti and Costa, 2011). Recently the *A. flexuosa* was employed with success in whole-sediment bioassays for determination of acute toxicity of samples from Guanabara Bay (Rio de Janeiro state, SW Brazil) in response to point and diffuse sources of pollution (Campos et al., 2019). Other investigations employed the clam to estimate the effects of diesel oil spills and changes in biochemical biomarkers related to water-soluble fraction of diesel oil (Sardi et al., 2017; Braga et al., 2018).

Recent studies reported changes of biomarkers in bivalves as endpoints to track the biological effects of dredging in Tropical ecosystems including Brazil, but they are limited to in situ approaches. The oyster *Crassostrea rhizophorae* was transplanted to sites of navigation channel during dredging activities at the Port of Santos (SW, Brazil) and accumulated metals in their whole-body tissues with changes on biomarkers responses observed in their gills (Maranho et al., 2012; Pereira et al., 2014). Similarly, *A. flexuosa* transplanted to Mucuripe harbor (NE Brazil) during sediment dredging also exhibited these effects (Moreira et al., 2019a), suggesting that the species is a potential candidate of test organism for studies on bioaccumulation and biomarkers not only in field exposures but also in toxicity bioassays.

In consideration of the need for alternative and new test organisms to be used in sediment quality and dredging materials assessments of tropical ecosystems, this study aimed to determine the ability of the clam *A. flexuosa* to respond in laboratory bioassays using whole-sediment exposures. Two surveys of sediment sampling were carried out in Mucuripe Bay and clams exposed to samples in laboratory. Contents of contaminants (metals and hydrocarbons) were quantified in whole-body tissues, while biochemical and effects biomarkers measured as sublethal endpoints.

2. Material and methods

2.1. Study area

The climate of Ceará state is typically semi-arid, being influenced by latitudinal variation of the Intertropical Convergence Zone (ITCZ). The temperatures are elevated (24 °C–30 °C), and E-SE trade winds blow constantly in region (4 m/s average), regulating the transport of sediments along the coastal zone (Jimenez et al., 1999; Paula et al., 2013). The seabed of the region is characterized by sandy sediments rich in up to 95% biogenic carbonates originated from calcareous algae (Marques et al., 2008), and terrigenous materials characterized by clays and siliciclastic particles, especially near the shore (Lacerda and Marins, 2006).

The study was conducted in the Mucuripe harbor, in the bay area of Ceará State capital, the city of Fortaleza. Mucuripe is one of the main port facilities of the Brazilian NE region, characterized by its access channel, and a 1,900 m long jetty. The occurrence of the jetty combined with the intense transport of particles and other organic and/or inorganic materials by the coastal currents results in an intense deposition within the access channel, which is mitigated by deepening dredging (Maia et al., 1998). Point and diffuse sources such as the urban runoff, harbor activities and the release of effluents from industrial (oil refinery) and domestic origin, have introduced different chemicals in the Bay. Previous studies reported sediment contamination (metals, PAHs and tributyltin), associated with toxicity, highlighting potential risks posed by dredging in the area (Moreira et al., 2017).

2.2. Sampling of organisms, sediments, and exposure design

Clams were collected manually in muddy to sandbanks, in the city of Icapuí (Ceará state), at Requenguela beach during the low tide (4°40′54.7″S, 37°20′13.9″W). Organisms were kept in thermal boxes during the transfer to the laboratory facilities and acclimated for 10 days until the experiments (clean seawater, temperature of 25 °C and salinity of 35 ‰). In the laboratory, clams were fed on *Chlorella* sp daily (approximately 0.4–0.6 g L-1 of dry weight). Ethical issues regarding animal sampling, handling, and experiments have been approved by the Chico Mendes Institute for Biodiversity Conservation (ICMBio) of the Brazilian Ministry of the Environment (SISBio license #21807-1).

The sampling surveys in Mucuripe harbor were set in two periods, at the intense dredging (survey 1: January 24, 2011), and at the end of it (survey 2: July 29, 2011). For sediment sampling, we selected three sites impacted directly by the sediment excavation of the Hopper Dredger: MD1, which is in front of the commercial docks, and MD2 by the oil terminal pier. The site MD3 is located outside the boundaries of harbor, by the signaling buoy of navigation channel (Figure 1). Sediments from Requenguela beach were also included as a reference sample for each survey set of exposure.

The sediment samples were obtained by using a *van Veen* grab sampler (0.026 m^2). Aliquots for toxicity tests were placed in refrigerated

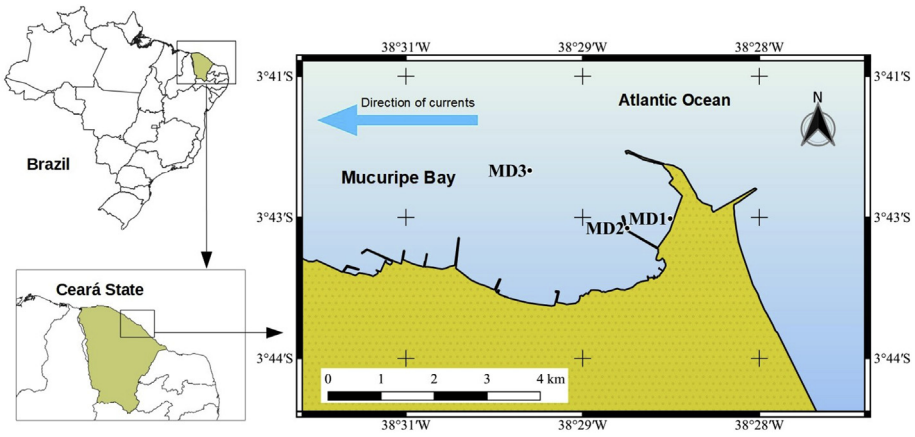

Figure 1. Location of sediment sampling sites in Mucuripe Bay, Fortaleza during the intense dredging (Survey 1) and after the end of activities (Survey 2). Mucuripe dredging sites 1 (MD1), 2 (MD2) and 3 (MD3). Reference site located at Icapuí.

coolers, transported, and stored in the laboratory at 4 °C. For the contaminant analyses of each sample, two subsamples were separated. One was dried at room temperature by using a desiccator cabinet and stored in plastic containers for subsequent analyses of inorganic chemicals and sediment grain size. The other one was wrapped into pre-cleaned aluminum foil and stored at -20 °C for the analysis of organic contaminants.

For the bioassays with *A. flexuosa*, each batch was assembled in triplicate per sample site, by using 5L glass bottles as exposure chambers containing 500 g of whole-sediment sample and 5L of clean and filtered seawater (45 μm, salinity 35‰). After the equilibration period (24h), 7 healthy organisms (juveniles, 15mm length) were introduced into each chamber and the system was kept under photoperiod (12h light: 12h dark), with constant aeration and temperature (25 ± 2 °C). The exposure time was set at 28 days, and four batches were prepared for different intervals of 7 days as a time factor (7, 14, 21, and 28). The other two groups from the acclimation conditions were sampled and designated as the control group for each survey. No mortality rates were observed and at each time of exposure, a batch was sampled, and the animals euthanized (ice-based method) for the dissection of whole-body soft tissues. Then, animals were sorted and tissues from 8 animals were pooled and freeze dried for the determinations of As and metals, and the other pool (n = 8) designated for hydrocarbons analysis. Tissues from the remaining organisms, assigned for biomarkers analysis (n = 5 for each biomarker), were frozen and kept at -70 °C until the analysis.

2.3. Analysis of sediments and bioaccumulation in whole-body tissues

Particle size fractions of sediments were measured using the wet sieving method to separate fine sediments (silt + clay), followed by dry sieving to determine sand fractions (Mccave and Syvitski, 1991). Calcium carbonate contents ($CaCO_3$) were removed with HCl and then, total organic carbon (TOC) levels were quantified in a TOC analyzer (model Shimadzu TOC-V, coupled with an SSM-5000A unit for solid sample combustion). For trace metals, samples were digested in HNO_3, H_2O_2, and HCl (3:1:1) (USEPA, 1996). Then, extracts were analyzed in a Flame Atomic Absorption Spectrometry (FAAS) (Shimadzu AA 6200) for Cd, Cr, Cu, Ni, Pb, and Zn.

Also, the following hydrocarbons were analyzed on freeze-dried samples of sediments: aliphatic (AHs: 26 compounds), polycyclic aromatic hydrocarbons (PAHs; 39 substances), and linear alkylbenzenes (LABs; 26 compounds). Hydrocarbons were extracted using a Soxhlet apparatus (n-hexane/dichloromethane (1:1, v/v)) (UNEP, 1991), and the extracts fractionated into F1 (AHs and LABs) and F2 (PAHs) via silica gel-alumina column chromatography. After that, AHs were quantified on a gas chromatography (GC) model 6890 from Agilent Technologies with flame ionization detector (GC-FID), while PAHs and LABs were quantified on GC coupled to a 5973N mass spectrometer (GC-MS) in a selected ion mode (SIM). On the parameters, temperatures were set as 280 °C for injection port, 300 °C for interface and 230 °C for ion source. Gas flux was set at 1 mL^{-1} min, electron impact was at 70 eV, and the mass range ranged from 50 to 550 amu.

Whole-body soft tissues were digested and analyzed for As and trace metals, according to procedures described in detail by Trevizani et al. (2016). Samples were extracted and elements quantified in an Inductively Coupled Plasma Optical Emission Spectrometry (ICPOES) Varian, 710-ES series. Similarly, procedures employed in the analysis of hydrocarbons are described in Moreira et al. (2019a). Tissues were extracted in a soxhlet apparatus and submitted to GC/MS for total concentrations of AHs, PAHs, and GC/MS in a selected ion mode for LABs.

Chemical analyses results are expressed as average values of 2 pseudoreplicates, with coefficient of variation below 10%. The validation of analytical methods for sediments was supported by the analysis of surrogates, blank samples, and the Certified Reference Materials (CRM) for metals (BCR® 667) and hydrocarbons (NIST® SRM® 1944). As for the bioaccumulation, the validation of methods for metals was assured by the results of CRMs selected for oyster (NIST® SRM® 1566b) and mussel samples (NIST® SRM® 2976) The results of CRMs analysis for metals are in Supplementary Material. As for hydrocarbon analysis, the CRM NIST® SRM® 1945 was used and results for f individual substances are presented as data in brief.

2.4. Biomarker analysis

We conducted the analysis of biochemical biomarkers in whole-body soft tissues of *A. flexuosa* and the methods are detailed in Moreira et al. (2019a), except for acetylcholinesterase (AChE). Individually samples of (n = 5) were disrupted in a buffer solution (pH 7.6) of NaCl (100 mol L−1), EDTA (0.1 mmol L^{-1}), dithiothreitol (0.1 mmol L^{-1}) and 1 mM PMSF (*phenylmethylsulfonyl fluoride*, 1 mmol L^{-1}). Tissues were centrifuged at 4 °C (15000 x g for 20 min), and the supernatant collected for the analysis of enzymatic activities, whereas the homogenates were collected for the quantification of lipid peroxidation (LPO) and DNA damage. Total concentrations of proteins in both fractions were estimated by the Bradford protein assay (Bradford, 1976), as means to normalize the data of each biomarker, which is necessary procedure for the statistical comparisons.

The enzymes Ethoxyresorufin O-deethylase (EROD) and Glutathione S-transferase (GST) were chosen as a proxy of Phase I (oxidation) and Phase II (conjugation) biotransformation reactions, respectively. EROD is a member of Cytochrome P450 monooxygenases (1A group), referred to CYP1A-like enzyme (Siebert et al., 2017), which was determined in

samples following the method proposed by Gagné and Blaise (1993). The results are expressed in pmol min mg^{-1} of total protein. The glutathione enzyme GST participates in the conjugation of reduced glutathione (GSH) to different substances. The activity GST was determined according to the method described by McFarland et al. (1999), and results were expressed as nmol min mg^{-1} of total protein.

The antioxidant enzymes glutathione peroxidase (GPx) and glutathione reductase (GR) were also measured to estimate oxidative stress. GPx plays a role in the catalysis of H_2O_2 to water by the oxidation of GSH to GSSG and GR was measured as the indicator of GSH regeneration (van der Oost et al., 2003). Both activities of GPx and GR were determined in samples according to McFarland et al. (1999) and results were expressed as nmol min $mg^{-1}$of total protein for GPx, and as pmol min mg^{-1} of total protein for GR.

Levels of LPO and DNA damage were selected as metrics of injuries caused by contaminant exposure that can lead to cell death (Regoli and Giuliani, 2014). Lipid Peroxidation (LPO) was estimated in terms of thiobarbituric acid reactive substances (TBARS), which are generated from lipid oxidation by reactive oxygen species (Girotti, 1998; Janero, 1990). LPO was measured in according to the TBARS method proposed by Wills (1987), and results expressed as μmol L^{-1} mg^{-1} of total protein. DNA damage was analyzed by means of the strand breaks formation following the alkaline precipitation method of the genomic DNA linked to nucleoproteins (Olive, 1988; Martín-Díaz et al., 2009). The results were expressed as μg^{-1} of DNA mg^{-1} of total protein.

The enzymatic activity of acetylcholinesterase (AChE) was evaluated as a neurotoxicity biomarker. The enzyme acetylcholinesterase (AChE) was assessed in the supernatants according to the method described in Monserrat et al. (2006). Samples were incubated in a solution medium (potassium phosphate buffer 0.1 M, pH 7.6) containing acetylcholine iodide (0.075 mol L^{-1}) and 5,5-dithio-bisnitrobenzene acid (DTNB, 10 mmol L^{-1}). The enzymatic activity was monitored for 20 min (4 min intervals) at 412 nm and results are expressed in μmol mL^{-1} mg^{-1} of protein.

2.5. Data analysis

The results obtained to biochemical biomarkers were assessed using the non-parametric permutational multivariate analysis of variance (PERMANOVA). The method was developed as multivariate analysis but it can be used analogue to traditional ANOVA, once it calculates p-values from pseudo-F values and random permutations instead of ANOVA assumptions, which are generated from tabulated p-values (Anderson, 2001; Anderson et al., 2008). For both surveys, differences in the responses observed in clams from control conditions (day 0) were compared to responses observed in those exposed at different times within each treatment (sediment samples). Also, responses at each time were compared to their respective time of the reference sample (Icapuí).

Significant “site”, “exposure time” and “survey”, "survey" vs.“site", "survey" vs.“exposure time”, "site" vs. "time", and "survey" vs. "site" vs. "time" were tested through a three-way crossed PERMANOVA with "survey (2 levels: 1 and 2), “site” (4 levels: Icapuí, MD1, MD2, and MD3) and “exposure time” (4 levels: 7, 14, 21 and 28 days) as fixed factors. PERMANOVA tests were performed on the Euclidean distance of data, and pairwise comparisons were determined when significant differences ($p < 0.05$) were observed following 999 permutations (unrestricted permutation of raw data). Data were analyzed using the software PRIMER® (version 6) with the additional add-on package PERMANOVA (Clarke and Gorley, 2006).

The Integrated biomarker response (IBR) was used as proposed by Beliaeff and Burgeot (2002), aiming to rank treatments according to the responses of all biomarkers for each exposure time in all samples, following the calculations described in Devin et al. (2014). Based on the changes of biomarkers responses (increase or decrease), data were plotted in 7 axis radial charts for each time of exposure following the sequence AChE, EROD, GPx, GR, GST, LPO, and DNA damage, considering only the IBR generated by this combination, from 720 possibilities of arrangements (Devin et al., 2014).

Associations between exposure (bioaccumulation) and effects (biochemical biomarkers expressed as IBR values) were observed through a Principal Components Analysis (PCA). A matrix was constructed containing data of IBR with total levels of metals (including As), and hydrocarbons. Data were submitted to a log (x+1) transformation to trim down the differences of variables scales. Then, the first three components were extracted based on 1000 bootstrap PCA and the cut-off for the component loading was set at |0.50| as a relevant correlation (Comrey and Lee, 1992).

3. Results and discussion

Data of the sediment analysis are exhibited in Table 1. Mucuripe Bay samples and reference were mostly sandy, with higher levels of fine sediments observed in MD1. The contents of TOC were low with increased values reported also in MD1. These characteristics of sediments within the bay are caused by the changes in the transport of materials along the coastal zone induced by the jetty at the harbor area, resulting thus in the high deposition of mud and organic matter (Maia et al., 1998; Paula et al., 2013). These findings corroborate the sedimentary facies described for the region with sandy sediments with mud deposition occurring at the coastal zone, as a result of harbor installations (Maia et al., 1998; Lacerda and Marins, 2006; Marques et al., 2008).

The contents of contaminants exhibited the same pattern of deposition in MD1. The reference sample was found to be sandy, with low content of TOC and exhibited lower contamination. Concentrations of Al, Cr, Zn, AHs, and PAHs were slightly higher in survey 1. Levels of LABs were detected only in MD1 during survey 2. Concentrations were compared to both threshold (Level 1) and probable effect (Level 2) benchmarks of the Federal normative #454 (Brasil, 2012), which are derive from SQGs applied in North America and Europe (Long et al., 1995; EC, 2008; HPA, 2011), and also with site-specific sediment quality values (SQV) calculated for the Estuarine System of Santos (Choueri et al., 2009). No SQGs exceedances were found, but concentrations of Pb in all samples (except reference) were above level 1 and 2 of SQVs, suggesting the potential risks of toxicity related to sediment contamination.

Prior to dredging activities, higher concentrations of Hg, Cd, Cu, Ni, Zn, and PAHs were observed in sediments of Mucuripe Bay (Buruaem et al., 2012, 2016). Contamination levels were higher compared to those found in this study and they were also associated with acute toxic effects on *T. viscana*, and with chronic effects of liquid phases observed on the larval development of *L. variegatus* (Moreira et al., 2017). The potential effects of the sediment samples from MD1 analyzed in this study were also characterized and results revealed acute toxicity of whole-sediment exposures on *T. viscana*, and *A. agilis*, while chronic effects determined in *T. biminiensis* (Moreira et al., 2019b). In the same investigation, liquid phases exhibited acute toxicity of sediment-water interface (SWI) on the mysid *Mysidopsis juniae*, and chronic effects of SWI and elutriates were observed on sea urchin larvae *L. variegatus*. Based on these results is possible to affirm that despite contamination levels have reduced as a result of dredging, the potential ecological risks of toxic effects at low levels still relevant.

Data of bioaccumulation measured in whole body soft tissues of *A. flexuosa* from sediment toxicity bioassay are given in Table 2. Clams exposed to the reference sample exhibited higher contents of chemicals (As, Zn, AHs, and PAHs) in relation to the control sample (animals from acclimation tank). Animals from MD1 and MD2 exhibited higher contents for most of the contaminants. Exposures to MD3 caused an uptake of As, Cd, Zn, and all hydrocarbons. For survey 2, clams from reference site exhibited elevated contents in relation to control only for LABs. Exposures to MD1 and MD2 resulted in high contents of As, and hydrocarbons. Animals from MD3 incorporated high amounts of As, Cd, Cu, Pb, AHs and LABs.

Table 1. Profile of physical and chemical characteristics of sediments collected in Mucuripe Bay, Fortaleza during the intense dredging (Survey 1) and after the end of activities (Survey 2). Reference site located at Icapuí. Exceedances marked in bold.

Variable	Reference	Survey 1			Survey 2			SGQs		SQVs	
		MD1	MD2	MD3	MD1	MD2	MD3	Level 1	Level 2	Level 1	Level 2
Bathymetry (m)	0	11	6	5	13	6	8	-	-	-	-
Sand (%)	95.9	37.9	82.7	91.6	36.6	57.6	87.9	-	-	-	-
Fine particles (%)	4.6	62.2	19.1	10.0	63.5	44.4	13.4	-	-	-	-
TOC (%)	0.10	0.49	0.07	0.06	0.69	0.04	0.06	10	-	-	-
Al (%)	0.09	0.96	0.24	0.25	0.96	0.06	0.08	-	-	-	-
Fe (%)	0.13	1.06	0.25	0.26	1.08	0.14	0.21	-	-	-	-
Cd (μg g^{-1})	0.19	0.16	0.16	0.15	0.15	0.14	0.14	1.2	7.2	-	0.75
Cr (μg g^{-1})	<2.0	25.9	15.5	13.4	2.5	<2.0	<2.0	81	370	-	65.8
Cu (μg g^{-1})	0.4	13.6	1.4	1.6	11.1	2.0	2.2	34	270	-	69
Ni (μg g^{-1})	2.1	***8.0**	1.6	2.2	***10.1**	***4.2**	3.5	20.9	51.6	3.89	21.2
Pb (μg g^{-1})	9.8	***22.0**	***15.9**	***19.4**	***22.9**	***13.9**	***22.4**	46.7	218	10.3	22
Zn (μg g^{-1})	0.7	25.8	2.5	2.8	21.0	1.1	2.1	150	410	37.9	110.4
ΣAHs (μg g^{-1})	0.6	655	1.0	0.8	408	0.5	0.5	-	-	-	-
ΣPAHs (ng g^{-1})	<1.0	***1160**	3.0	<1.0	***691**	<1.0	<1.0	4000	-	163	950
ΣLABs (ng g^{-1})	<0.8	<0.8	<0.8	<0.8	91.6	<0.8	<0.8	-	-	-	-

Bathymetry (m): underwater depth; TOC: total organic carbon; AHs: aliphatic hydrocarbons, PAHs: polycyclic aromatic hydrocarbons; and LABs: linear alkylbenzenes. * = SQVs exceedances.

Most of treatments exhibited monotonic responses, especially for the hydrocarbons in MD1 (surveys 1 and 2) and MD3 (survey 2). Relevant sources of hydrocarbons including volatile compounds were related to harbors activities, effluent from an oil refinery and the inputs from the drainage system of urban runoff (Cavalcante et al., 2010; Buruaem et al., 2016). Levels of inorganic chemicals found in this study were like those found in *A. flexusoa* sampled in the estuaries of Potengi and Curimataú rivers, both located in Rio Grande do Norte state (NE Brazil) and affected by urban activities (Silva et al., 2006). Animals from the same species from impacted sites of Todos os Santos Bay (state of Bahia, NE Brazil) also exhibited similar concentrations of metals (Cd, Cu, Ni, and Zn) in their whole-body tissues (Jesus et al., 2008). Regarding the organic compounds, concentrations of PAHs are higher than those observed for the total of 16 EPA PAHs detected in tissues of calms (*A. flexuosa*) submitted to in situ experiments involving sediment banks spiked with marine diesel oil (Sardi et al., 2017).

As for the impacts of dredging activities, *A. flexuosa* was also used in a recent study as a model to observe the release of chemicals to water layer (metals and hydrocarbons) from sediment resuspension of Mucuripe Bay using in situ exposures (Moreira et al., 2019a). Animals were transplanted to the same locations of MD1 and MD2 for 28 days and exhibited high levels of Cu, Zn, PAHs, and LABs, confirming the potential of species to incorporate and accumulate contaminants. Based on these results we consider the *A. flexuosa* a suitable organism for bioaccumulation studies in marine sediments from tropical regions.

PERMANOVA results based on biomarker responses are presented in Supplementary Material, and we focused on pointed out the results only for the factors time and site. Changes in enzymatic activities of phase I and II, antioxidant responses, AChE, LPO, and DNA strand breaks were monitored over time in individual clams as a complementary line of evidence of sediment quality in order to assess the potential effects of dredged materials from Mucuripe bay and establish cause-and-effects relationships (Chapman, 2007). We used whole-body soft tissue due to the reduced size of the organisms and the limited amount of tissue. Such an approach has been successfully applied to identify sublethal responses of other invertebrates such as gastropods (Sarkar et al., 2014), mussels (Galloway et al., 2002), amphipods (Maranho et al., 2015; Moreira et al., 2016), and polychaete worms (Maranho et al., 2014; Saes et al., 2019).

For phase I enzyme, clams exposed to samples collected in Survey 1 exhibited no significant change of EROD enzymatic activity compared to control conditions (day 0). A decreased activity was found for MD2 in the day 21 compared with the respective time of the reference sample. In the survey 2, samples exhibited no change in relation to control, but an increase of EROD occurred in MD2 (day 28) compared with the reference sample. The GST activity for survey 1 increased in all exposure times of MD1, and in day 7 of reference sample (Icapuí) and MD3, when compared with control. Comparison with the reference sample exhibited increased in GST only for MD1 (days 7 and 28). In the survey 2, all samples (on day 28) presented increased activity compared with control, while GST were high in MD1 and MD3 on day 28 compared to the reference. Decreased activities of GST were found on day 1 of MD2 and MD3 (Figure 2).

The results observed for phase I and II enzymes suggest that biotransformation via CYP1A-like enzymes were not activated. These results are different to those reported by Moreira et al. (2019a) for *A. flexuosa* caged in the same location of MD1 and exhibited an increased activity of EROD in tissues of gills. At the same time, the results for GST activity in clams exposed to sediments in the laboratory were more consistent with GST responses determined in gills and digestive clams of animals from the study mentioned above. Such metabolism is likely activated as a detoxification response of metals, organic substances, and contaminants of emerging concern and protection from oxidative stress induced by phase I by-products (Coppock and Dziwenka, 2014). Since animals presented elevated levels of hydrocarbons after 28 days, it is possible that other CYP enzymes may be participating in the biotransformation process of organic substances in clams during laboratory exposures.

Elevated activities of GPx were observed in *A. flexuosa* exposed to MD1 (days 14–28), MD2 (day 28) and MD3 (day 21) compared to control of survey 1, while only MD1 was significant in comparison to the reference. For survey 2 all samples presented increase of GPx at day 28, and both organisms from reference and MD2 presented higher values at day 7 in comparison to control, while samples MD1 to MD3 (day 28, and day 21 for MD1) exhibited higher activity of GPx in relation to reference. Regarding GR activities in survey 1, increases were detected in the animals from MD1 (days 21 and 28) and MD3 (day 28), while in those from MD2 the activity decreased (days 14 and 21). The GR activities reported in MD1 (days 21 and 28) and MD2 (day 21) were also significantly different from the reference. For survey 2 GR increased in clams from MD1 to MD3 (day 28) compared to both control and reference, while samples from MD3 (day 7) were also different from the reference (Figure 3).

Table 2. Chemical profile based on concentrations of metals and hydrocarbons measured in whole-body tissues of *A. flexuosa* exposed to sediments from Mucuripe Bay. Values expressed in dry weight. Reference site located at Icapuí.

Sample	Time	As (μg g^{-1})	Cd (μg g^{-1})	Cr (μg g^{-1})	Cu (μg g^{-1})	Ni (μg g^{-1})	Pb (μg g^{-1})	Zn (μg g^{-1})	ΣAHs (μg g^{-1})	ΣPAHs (ng g^{-1})	ΣLABs (ng g^{-1})
Survey 1											
Control	Day 0	4.1	0.5	0.7	10.1	1.2	<0.2	29.1	8.6	195.0	271.7
Reference	Day 7	6.7	0.7	0.4	9.3	0.6	<0.2	37.7	15.9	87.1	290.9
Reference	Day 14	9.0	0.8	0.9	9.5	1.4	<0.2	57.5	13.9	84.7	504.1
Reference	Day 21	6.1	0.5	0.6	16.4	0.9	<0.2	44.4	15.7	135.0	511.2
Reference	Day 28	5.2	0.7	1.3	8.1	0.7	<0.2	57.9	17.4	198.6	327.5
MD1	Day 7	5.4	0.6	0.5	22.5	0.6	<0.2	38.5	24.1	271.1	664.2
MD1	Day 14	5.6	0.7	0.5	23.9	0.6	<0.2	39.2	17.1	389.3	762.7
MD1	Day 21	6.1	0.6	0.7	11.0	1.1	<0.2	48.7	26.0	446.0	882.4
MD1	Day 28	6.1	0.6	1.2	12.2	1.5	0.5	50.7	28.9	468.2	801.8
MD2	Day 7	5.8	0.5	0.6	12.3	1.2	<0.2	40.9	15.9	369.2	518.1
MD2	Day 14	5.7	0.8	0.4	9.1	0.9	<0.2	40.1	15.3	269.5	751.2
MD2	Day 21	5.9	1.1	3.2	9.9	1.0	<0.2	44.7	17.5	263.2	424.5
MD2	Day 28	5.6	0.8	0.7	12.3	1.2	0.5	46.3	18.2	376.6	517.9
MD3	Day 7	4.8	0.6	0.7	13.2	0.9	<0.2	36.1	10.8	241.0	722.4
MD3	Day 14	5.6	0.8	0.5	10.2	0.8	<0.2	42.2	16.6	252.4	598.2
MD3	Day 21	7.3	0.9	1.0	10.4	0.7	<0.2	45.6	14.9	246.2	448.0
MD3	Day 28	6.3	2.6	0.5	13.1	1.2	<0.2	45.2	21.9	221.9	551.3
Survey 2											
Control	Day 0	5.4	1.0	0.4	8.9	1.0	<0.2	39.4	9.4	132.9	267.7
Reference	Day 7	4.9	0.9	0.5	9.2	0.7	<0.2	42.6	14.6	196.9	553.6
Reference	Day 14	7.0	0.7	0.6	13.3	0.8	<0.2	43.1	10.4	152.3	415.6
Reference	Day 21	7.9	1.4	0.8	9.6	0.7	<0.2	42.4	15.4	177.5	470.2
Reference	Day 28	6.7	0.7	0.5	9.7	0.7	0.3	41.0	12.2	154.1	380.5
MD1	Day 7	5.2	0.8	0.4	8.6	0.7	0.3	35.6	15.4	450.8	668.3
MD1	Day 14	6.3	0.7	0.4	11.0	0.6	<0.2	42.8	17.3	290.8	767.2
MD1	Day 21	4.9	1.0	0.4	6.7	0.5	<0.2	35.7	10.1	293.8	855.4
MD1	Day 28	7.4	1.2	0.5	8.5	0.8	<0.2	41.9	22.3	291.1	1204.9
MD2	Day 7	5.9	1.9	0.3	13.4	0.6	<0.2	39.5	14.3	160.7	496.6
MD2	Day 14	5.8	0.8	0.4	8.6	0.5	<0.2	42.5	24.4	404.7	679.6
MD2	Day 21	5.8	0.9	0.5	11.9	0.6	0.6	45.3	16.5	329.4	735.2
MD2	Day 28	5.6	0.9	0.6	9.2	0.8	<0.2	42.4	23.6	401.3	1049.6
MD3	Day 7	6.9	1.0	0.5	8.2	0.6	<0.2	40.4	16.3	208.7	465.5
MD3	Day 14	5.7	0.6	0.5	30.2	0.9	<0.2	47.0	9.5	308.6	596.3
MD3	Day 21	4.6	0.9	0.5	10.9	<0.4	<0.2	39.5	11.6	137.0	769.6
MD3	Day 28	6.7	1.7	0.5	14.9	0.7	1.3	45.4	14.4	144.5	779.4

In the study mentioned earlier, *A. flexuosa* and the oysters *C. rhizophorae* exposed to resuspended sediments in Mucuripe Bay also exhibited high activities of GR and GPx in their gills and digestive glands tissues (Moreira et al., 2019a). These results contrast with previous investigations that reported no changes in antioxidant responses (including GPx activity), in gills and digestive glands of *A. flexuosa* sampled in sites impacted by contamination sources in Paranaguá estuarine system (SW Brazil) (Sardi et al., 2016) and also in mudflats spiked with diesel oil in spills simulations (Sardi et al., 2017). Thus, we consider that clam responded to oxidative stress induced by dredged materials.

Increased levels of LPO in relation to control were detected in *A. flexuosa* exposed to sediments from MD1 (day 7), MD2 (day 7) and MD3 (day 28) collected in survey 1, and levels found in MD1 and MD3 were different to those measured in the reference organisms. A decrease of LPO was observed in MD3 (day 21) in comparison to reference. In calms exposed to samples from survey 2, LPO increased in reference (day 14), MD1 (days 7 and 14), MD2 (day 14), and MD3 (days 7 and 28) compared to control. LPO levels decreased in samples from reference (days 21 and 28). Such decreases resulted in differences in MD1 (day 21), MD2 (day 21 and 28), and MD3 (days 7, 21 and 28) (Figure 3).

In aquatic bivalves, LPO can be generated as a result of by oxidative stress and by chemicals present in the medium (water or sediments), posing risks to membrane lipids of the cells (Almeida et al., 2007). Since the levels of chemicals in sediment samples were minimal to moderate according to SQGs (Brasil, 2012), but metals and hydrocarbon were available and uptaken by clams, it is possible to relate these effects to a lower status of sediment contamination. However, the increase followed by the decrease of TBARs levels in the reference sample of survey 2 may also indicate a natural variation of biomarkers, as pointed out by other studies (van der Oost et al., 2003; Sardi et al., 2017).

The activity of AChE in survey 1 was induced in clams from reference samples (days 14 and 21), MD1 (days 14 and 21), MD2 (days 7 and 14), and MD3 (days 7,14, and 28). The AChE results of MD1 (days 14 and 21), MD2 (day 7), and MD3 (days 7 and 28) were different from their respective exposure time of the reference sample. Significant decreases from reference were also found for MD2 and MD3 on day 21. In the survey 2, reference (days 7 and 28), MD1 (days 14–28), MD2 (days 7, 14 and 28), and MD3 (days 7–28) were increased compared to control, while only MD1 (day28) exhibited induced activity in relation to reference samples (Figure 4).

The enzyme AChE is responsible for the hydrolysis of the neurotransmitter acetylcholine forming thus acetate and thiocholine, causing the cholinergic receptor to return to an initial condition, playing an important role in many physiological functions (Andreescu and Marty,

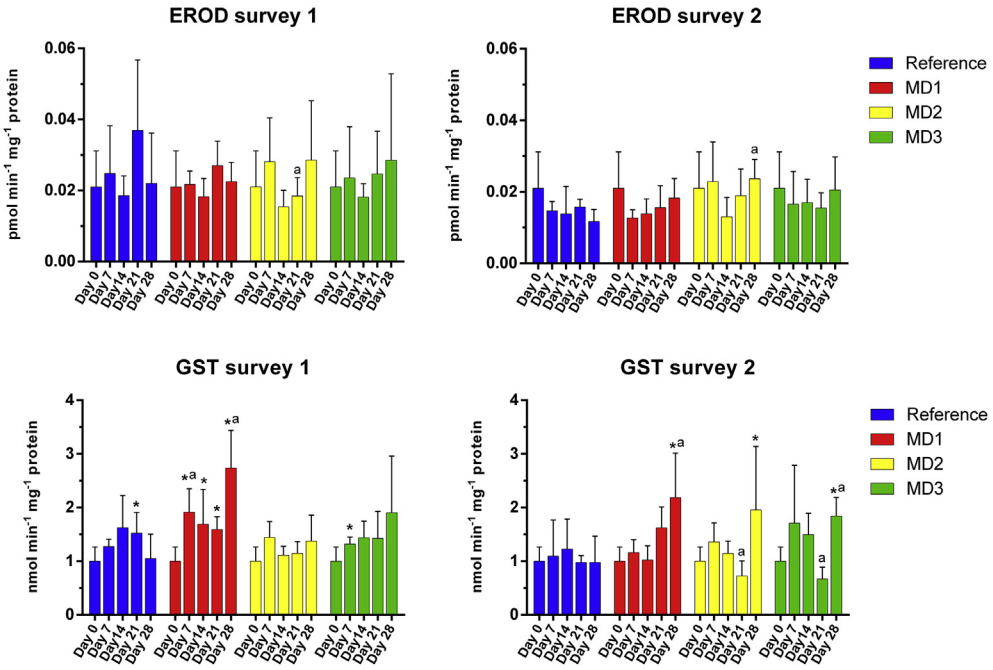

Figure 2. Biomarkers responses monitored in tissues of the clam *Anomalocardia flexuosa* exposed to sediments from Mucuripe Bay during dredging operations. Phase I and II enzymes measured by means of the activities of Ethoxyresorufin-O-deethylase (EROD) and Glutathione S-Transferase (GST). Bars represent the mean +SD (n = 5). * = Pairwise comparisons of exposure times in each treatment with day 0; a = Pairwise comparisons of exposure times (except day 0) in each treatment with the respective time of reference site (PERMANOVA, $p < 0.05$).

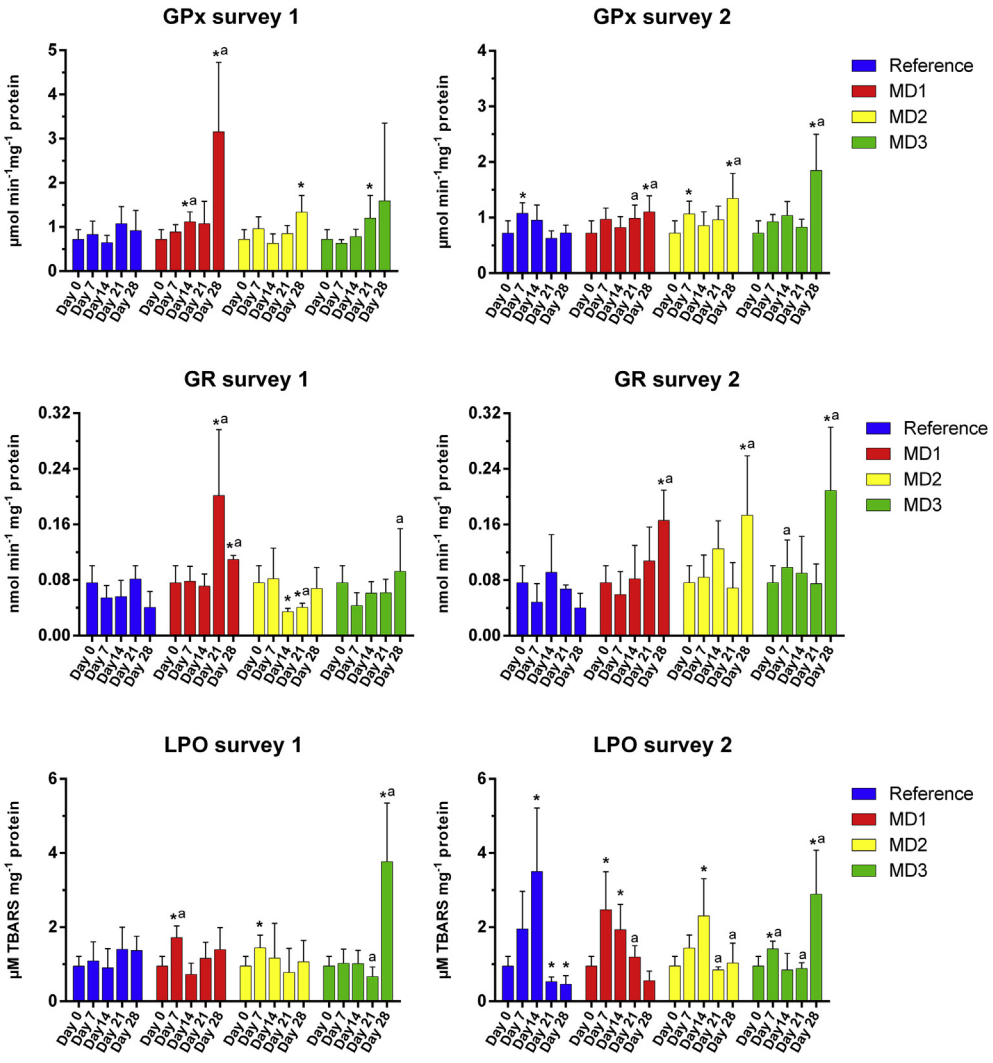

Figure 3. Biomarkers responses monitored in tissues of the clam *Anomalocardia flexuosa* exposed to sediments from Mucuripe Bay during dredging operations. Activities of antioxidant enzymes measured by means of the Glutathione Peroxidase (GPx) and Glutathione Reductase (GR). Lipid Peroxidation (LPO) measured as thiobarbituric acid reactive substances (TBARs). Bars represent the mean +SD (n = 5). * = Pairwise comparisons of exposure times in each treatment with day 0; a = Pairwise comparisons of exposure times (except day 0) in each treatment with the respective time of reference site (PERMANOVA, $p < 0.05$).

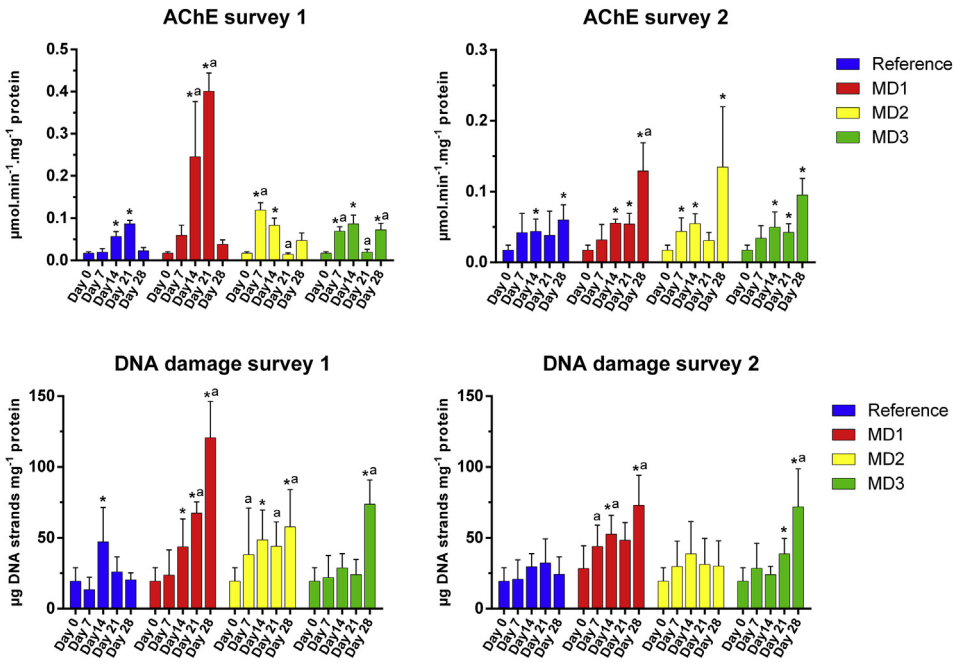

Figure 4. Biomarkers responses monitored in tissues of the clam *Anomalocardia flexuosa* exposed to sediments from Mucuripe Bay during dredging operations. Neurotoxicity effect by means of the acetylcholinesterase enzymatic activity (AChE). DNA damage measured as DNA strand breaks. Bars represent the mean +SD (n = 5). * = Pairwise comparisons of exposure times in each treatment with day 0; a = Pairwise comparisons of exposure times (except day 0) in each treatment with the respective time of reference site (PERMANOVA, $p < 0.05$).

2006). Inhibition of AChE has been often associated with neurotoxic effects of organophosphate and carbamate pesticides, but other authors have also reported enhanced activity of AChE in brain tissues of the fishes *Anabas testudineus* and *Heteropneustes fossilis* treated with glyphosate (Samanta et al., 2014), and in muscle of *Leporinus obtusidens* exposed to clomazone, propanil and metsulfuron methyl (Moraes et al., 2007). It was hypothesized that AChE activation occurred as a response to the accumulation of acethylthiocholine caused by the contaminant, resulting in the overstimulation of the receptors and induction of LPO, which can also lead to injuries in the cell. Also, increased expression and activity of the AChE as a response to stress have been reported in apoptotic cells (Masha'our et al., 2012; Zhang and Greenberg, 2012).

For DNA damage, samples from survey 1 exhibited elevated amounts of strands compared to control conditions in tissues from reference (day 14), MD1 (days 7–28), MD2 (days 14 and 28), and MD3 (day 28). Results of MD1 (days 21 and 28), MD2 (days 7, 21 and 28), and MD3 (day 28) were also different from the reference. In survey 2, the damage in DNA was high in clams from MD1 (days 14 and 28) and MD3 (days 21 and 28) compared to control, while results from MD1 (days 7, 14 and 28) and MD3 (day 28) were different to those found for the reference sample (Figure 4). Similar responses for DNA strand breaks were measured in tissues of *A. flexuosa* (gills and digestive glands) transplanted to MD1 and MD2 during dredging activities (Moreira et al., 2019a). Induction of DNA strands was also reported in tissues samples (gills and digestive glands) of the clam *Ruditapes philippinarum* transplanted to contaminated areas of Santander Bay (Ramos-Gómez et al., 2011), corroborating our results. Strand breaks of DNA are an effective parameter to be assessed in bivalves for indicating genotoxicants occurrence in the environment such as metals and hydrocarbons (Lam, 2009), or as an outcome of oxidative stress as also reported in this study. The long-term outcomes of genotoxic effects include reproductive impairment, abnormal development, lethal mutations, and changes in the genetic variability (Dickmann et al., 2004).

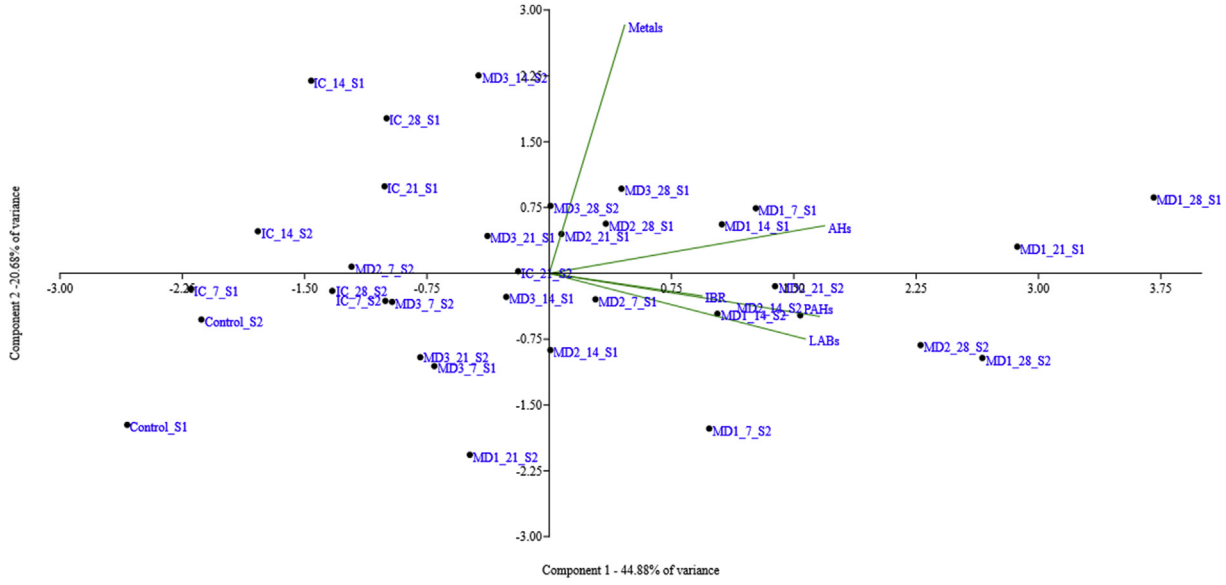

Figure 5. 2D ordination based on PCA results of Biomarkers responses and the concentrations of contaminants in whole-body tissues of *Anomalocardia flexuosa* exposed to sediment samples of Mucuripe bay for 7, 14, 21, and 28 days.

Results of the IBR index based on effects measured in *A. flexuosa* are presented in the Supplementary Material. For survey 1, the high IBR values ranked samples according to such distribution: MD1 (day 28), MD2 (day 21), MD3 (day 21), and Icapuí (day 21). For survey 2, the IBR exhibited a different pattern, due to the elevated values compared to survey 1, including control: Icapuí (day 21), MD2 (day 21), MD1 (day 28) and MD3 (day 28). IBR has been employed to ranking samples by integrating responses of the clams *R. philippinarum* treated with liquid extracts of sediments from Jiaozhou Bay (China) (Lin et al., 2018), and *Chione elevata* collected in sediments from different sites along the coastal zone of Laguna Madre (Mexico) (Aguilera et al., 2019). In this study, the IBR results revealed that after 28 days, clams treated with sediments from the sites MD1 and MD3 of survey 1 were more affected than animals from control, while clams exposed to samples from survey 2 exhibited a similar pattern, except for the effects observed also in the reference site. In the case, the result can be attributed to the arranging of the biomarkers in the radial plots, especially LPO followed by DNA damage, which also varied over time in such a sample, influencing thus in the area of the triangle (Devin et al., 2014).

PCA results are shown in the Supplementary Material. The first three components accounted for 83.08% of total variance. Axis 1 (44.88% of variance) correlated concentrations of hydrocarbons (AHs, PAHs, and LABs) with biomarkers' responses (IBR). Levels of metals were correlated negatively to axis 2 (20.68% of variance), while IBR values were correlated positively to axis 3 (17.51% of variance). The 2-D ordination of components 1 and 2 clustered samples from MD1 and MD2 (surveys 1 and 2), from control, reference sites and MD3 (Figure 5).

PCA correlated changes in biomarkers with organic contaminants that accumulated by the clams, suggesting that the impaired health effects may be induced by the hydrocarbons presented in sediment samples. Correlations pattern between biomarkers and hydrocarbons were observed for *A. flexuosa* and the oyster *C. rhizophorae* caged during sediment resuspension in Mucuripe Bay (Moreira et al., 2019a). In this study, responses included phase II and antioxidant enzymes which representing a defense mechanism to deal with contaminants (Coppock and Dziwenka, 2014). Increased DNA damage and LPO also represent risks of both cytotoxicity and genotoxicity (Almeida et al., 2007; Moreira et al., 2019a) induced by the contaminants associated to dredged materials.

4. Conclusions

The clam *A. flexuosa* responded over time to laboratory exposures of sediments collected in Mucuripe Bay during the dredging activities, which qualify the organism as suitable organism to estimate the quality of sediments and dredged materials, by using bioaccumulation and biomarkers as complementary LOEs. Hydrocarbons were the chemicals of environmental concern and the main sources included harbor activities, industrial effluents, and inputs from urban runoff. The results also showed that SQGs are unable to predict bioaccumulation and subchronic effects (at biochemical level), suggesting that protective levels could be lower and that more studies are necessary to estimate new SQGs based on such LOEs. We also recommend that studies aimed to assess the risks of dredging and disposal of its materials should include responses of different tissues and parameters at organism and sub-organism levels (such as those studied here and others like the condition factor or neutral red retention time), which can be integrated into an approach regarding multilevel of biological organization.

Declarations

Author contribution statement

Lucas Buruaem Moreira: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Silvio Tarou Sasaki, Satie Taniguchi, Tiago Farias Peres, Rubens Cesar Lopes Figueira, Márcia Caruso Bícego, Rozane Valente Marins, Leticia Costa-Lotufo: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Denis Moledo de Souza Abessa: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico 573.601/2008-9. Dr. Lucas Moreira was supported by Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico 1571/07 & BMD-0008-00058.01.18/09, and Conselho Nacional de Desenvolvimento Científico e Tecnológico 142002/2010-0. Denis Moledo de Souza Abessa was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico 552299/2010-3 & 311609/2014-7.

Competing interest statement

The authors declare no conflict of interest.

Additional information

Supplementary content related to this article has been published online at https://doi.org/10.1016/j.heliyon.2020.e04030.

Acknowledgements

We thank the Companhia Docas do Ceará (port authorities of Ceará State) and Continent-Ocean Materials Transfer program (INCT-TMCOcean) of the Brazilian National Research Council (CNPQ) for the support. We also thank Dr. João E.P. de Freitas for assistance with sediment sampling.

References

ABNT, 2006. NBR 15350. Ecotoxicologia aquática – Toxicidade crônica de curta duração – Método de ensaio com ouriço do mar (Echinodermata: Echinoidea). Associação Brasileira de Normas Técnicas, Rio de Janeiro, Brazil.

ABNT, 2008. NBR 15638. Qualidade de água – Determinação da toxicidade aguda de sedimentos marinhos ou estuarinos com anfípodos. Associação Brasileira de Normas Técnicas, Rio de Janeiro, Brazil.

Aguilera, C., Leija, A., Torres, M., Mendoza, R., 2019. Assessment of environmental quality in the tamaulipas Laguna Madre, gulf of Mexico, by integrated biomarker response using the cross-barred venus clam *Chione elevata*. Water Air Soil Pollut. 230, 27.

Almeida, E.A., Bainy, A.C.D., Loureiro, A.P.M., Martinez, G.R., Miyamoto, S., Onuki, J., Barbosa, L.F., Garcia, C.C.M., Prado, F.M., Ronsein, G.E., Sigolo, C.A., Brochini, C.B., Martins, A.M.G., Medeiros, M.H.G., Mascio, P., 2007. Oxidative stress in *Perna perna* and other bivalves as indicators of environmental stress in the Brazilian marine environment: antioxidants, lipid peroxidation and DNA damage. Comp. Biochem. Physiol. A 146, 588–600.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis ofvariance. Aust. J. Ecol. 26, 32–43.

Anderson, M.J., Gorley, R.N., Clarke, K.R., 2008. PERMANOVA for PRIMER: Guide Tosoftware and Statistical Methods. Primer E Ltd, Plymouth, United Kingdom, p. 214.

Andreescu, S., Marty, J.-L., 2006. Twenty years research in cholinesterase biosensors: from basic research to practical applications. Biomol. Eng. 23, 1–15.

Araújo-Castro, C.M.V., Souza-Santos, L.P., Torreiro, A.G.A.G., Garcia, K.S., 2009. Sensitivity of the marine benthic copepod *Tisbe biminiensis* (Copepoda, Harpacticoida) to potassium dichromate and sediment particle size. Braz. J. Oceanogr. 57, 33–41.

Beliaeff, B., Burgeot, T., 2002. Integrated biomarkers response: a useful tool for ecological risk assessment. Environ. Toxicol. Chem. 21, 1316–1322.

Bradford, M.B., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein–dye binding. Anal. Biochem. 72, 248–254.

Braga, M.A., Brauko, K.M., Vicentini, M., Salgado, L.D., Assis, H.C.S., Dolatto, R.G., Grassi, M.T., Sandrini-Neto, L., Lana, P.C., 2018. Cytotoxicity and enzymatic biomarkers as early indicators of benthic responses to the soluble-fraction of diesel oil. Ecotoxicol. Environ. Saf. 164, 21–31.

Brasil, 2012. Resolution No. 454/12. Establishing General Guidelines to the Evaluation of Dredging Material in Brazilian Jurisdictional Waters, CONAMA. Environmental National Council.
Burton Jr., G.A., 2002. Sediment quality criteria in use around the world. Limnology 3, 65–75.
Buruaem, L.B., Taniguchi, S., Sasaki, S.T., Bícego, M.C., Costa-Lotufo, L.V., Abessa, D.M.S., 2016. Hydrocarbons in surface sediments of harbor areas in a tropical region (Ceará State, Northeast Brazil). Environ. Earth Sci. 75, 642.
Buruaem, L.M., Hortellani, M.A., Sarkis, J.E., Costa-Lotufo, L.V., Abessa, D.M.S., 2012. Contamination by metals of port zones sediments from Large Marine Ecosystems of Brazil. Mar. Pollut. Bull. 64, 479–488.
Campos, B.G., Moreira, L.B., Pauly, G.F.E., Cruz, A.C.F., Monte, C.N., Silva, L.I.D., Rodrigues, A.P.C., Machado, W., Abessa, D.M.S., 2019. Integrating multiple lines of evidence of sediment quality in a tropical bay (Guanabara Bay, Brazil). Mar. Pollut. Bull. 146, 925–934.
Cavalcante, R.M., Andrade, M.V.F., Marins, R.V., Oliveira, L.D.M., 2010. Development of a headspace-gas chromatography (HS-GC-PIDFID) method for the determination of VOCs in environmental aqueous matrices: optimization, verification and elimination of matrix effect and VOC distribution on the Fortaleza Coast, Brazil. Microchem. J. 96, 337–343.
Chapman, P.M., 2007. Determining when contamination is pollution — weight of evidence determinations for sediments and effluents. Environ. Int. 33, 492–501.
Chapman, P.M., Hollert, H., 2006. Should the sediment quality triad become a tetrad, a pentad, or possibly even a hexad? J. Soils Sediments 6, 4–8.
Choueri, R.B., Cesar, A., Abessa, D.M.S., Torres, R.J., Morais, R.D., Riba, I., Pereira, C.D.S., Nascimento, M., Mozeto, A.A., DelValls, A.T., 2009. Development of site-specific sediment quality guidelines for North and South Atlantic littoral zones: comparison against national and international sediment quality benchmarks. J. Hazard Mater. 170, 320–331.
Clarke, K.R., Gorley, R.N., 2006. Primer V6: User Manual/Tutorial. PRIMER-E, Plymouth.
Comrey, A.L., Lee, H.B., 1992. Afirst Course in Factor Analysis, second ed. Lawrence Erlbaum Associates, Publishers, Hillsdale, New Jersey, p. 488p.
Coppock, R.W., Dziwenka, M.M., 2014. Chapter 37 - biomarkers of petroleum products toxicity. In: Gupta, R.C. (Ed.), Biomarkers in Toxicology. Academic Press, Cambridge, pp. 647–654.
DeForest, D.K., Brix, K.V., Adams, W.J., 2007. Assessing metal bioaccumulation in aquatic environments: the inverse relationship between bioaccumulation factors, trophic transfer factors and exposure concentration. Aquat. Toxicol. 84, 236–246.
Devin, S., Burgeot, T., Giambérini, L., Minguez, L., Pain-Devin, S., 2014. The integrated biomarker response revisited: optimization to avoid misuse. Environ. Sci. Pollut. Res. 21, 2448–2454.
Dickmann, M., Waldmann, P., Schnurstein, A., Grummt, T., Brundbeck, T., Nagel, R., 2004. On the relevance of genotoxicity for fish populations II: genotoxic effects in zebrafish (*Danio rerio*) exposed to 4-nitroquinoline-1-oxide in a complete life-cycle test. Aquat. Toxicol. 68, 27–37.
EC, 2008. Criteria for the assessment of sediment quality in quebec and application frameworks: prevention, dredging and remediation. Ministère du Développement durable, de l'Environnement et des Parcs du Québec.
Gagné, F., Blaise, C., 1993. Hepatic metallothionein level and mixed function oxidase activity in fingerling rainbow trout (*Oncorhynchus mykiss*) after acute exposure to pulp and paper mill effluents. Water Res. 27, 1669–1682.
Galloway, T.S., Millward, N., Browne, M.A., Depledge, M.H., 2002. Rapid assessment of organophosphorous/carbamate exposure in the bivalve mollusc *Mytilus edulis* using combined esterase activities as biomarkers. Aquat. Toxicol. 61, 169–180.
Girotti, A.W., 1998. Lipid hydroperoxide generation, turnover, and effector action in biological systems. J. Lipid Res. 39, 1529–1542.
Hagger, J.A., Jones, M.B., Leonard, D.R., Owen, R., Galloway, T.S., 2006. Biomarkers and integrated environmental risk assessment: are there more questions than answers? Integrated Environ. Assess. Manag. 2, 312–329.
Hampel, M., Blasco, J., Martín Díaz, M.L., 2016. Chapter 5 - biomarkers and effects. In: Blasco, J., Chapman, P.M., Campana, O., Hampel, M. (Eds.), Marine Ecotoxicology: Current Knowledge and Future Issues. Academic Press, Cambridge, pp. 121–165.
HPA, 2011. Assessment Criteria for Dredged Material with Special Focus on the North Sea Region. Hamburg Port Authority. https://sednet.org/download/Dredged_Material_Criteria_North_Sea_0611.pdf. (Accessed 10 January 2020).
Janero, D., 1990. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. Free Radical Biol. Med. 9, 515–540.
Jesus, T.B., Fernandez, L.G., Queiroz, A.F.S., 2008. Avaliação da concentração de cádmio, cobre, ferro, manganês, níquel e zinco em *Anomalocardia brasiliana* (Gmelin, 1791) provenientes de zonas de manguezal da região de São Francisco do Conde e Madre de Deus, Recôncavo Baiano, BA. J. Braz. Soc. Ecotoxicol. 3, 77–84.
Jimenez, J., Maia, L.P., Serra, J., Morais, J., 1999. Aeolian dune migration, NE Brazil. Sedimentology 46, 689–701.
Lacerda, L.D., Marins, R.V., 2006. Geoquímica de sedimentos e o monitoramento de metais na plataforma continental nordeste oriental do Brasil. Geochim. Bras. 20, 123–135.
Lam, P.K.S., 2009. Use of biomarkers in environmental monitoring. Ocean Coast Manag. 52, 348–354.
Lin, Y., Liu, Q., Meng, F., Lin, Y., Du, Y., 2018. Integrated toxicity evaluation of metals in sediments of Jiaozhou Bay (China): based on biomarkers responses in clam *Ruditapes philippinarum* exposed to sediment extracts. Mar. Pollut. Bull. 131, 180–190.
Long, E.R., MacDonald, D.D., Smith, S.L., Calder, F.D., 1995. Incidence of adverse biological effects within ranges of chemical concentrations in marine and estuarine sediments. Environ. Manag. 19, 81–97.
Lotufo, G.R., Abessa, D.M.S., 2002. Testes de toxicidade com sedimentos total e água intersticial estuarinos utilizando copépodos bentônicos. In: Nascimento, I.,A., Sousa, E.,C.,P.,M., Nipper, M.,G. (Eds.), Métodos em Ecotoxicologia Marinha: Aplicações no Brasil, Artes Gráficas e Indústria Ltda, São Paulo, pp. 51–162.
Maia, L.P., Jimenez, J.A., Serra, J., Morais, J.O., 1998. The coastline of Fortaleza city. A product of environmental impacts caused by the Mucuripe harbor. Arq. Cienc. Mar 31, 93–100.
Maranho, L.A., Baena-Nogueras, R.M., Lara-Martín, P.A., DelValls, T.A., Martín-Díaz, M.L., 2014. Bioavailability, oxidative stress, neurotoxicity and genotoxicity of pharmaceuticals bound to marine sediments. The use of the polychaete *Hediste diversicolor* as bioindicator species. Environ. Res. 134, 353–365.
Maranho, L.A., Moreira, L.B., Baena-Nogueras, R.M., Lara-Martín, P.A., DelValls, T.A., Martín-Díaz, M.L., 2015. A candidate short-term toxicity test using *Ampelisca brevicornis* to assess sublethal responses to pharmaceuticals bound to marine sediments. Arch. Environ. Contam. Toxicol. 68, 237–258.
Maranho, L.A., Pereira, C.D.S., Choueri, R.B., Cesar, A., Gusso-Choueri, P.K., Torres, R.J., Abessa, D.M.S., Morais, R.D., Mozeto, A.A., DelValls, T.A., Martín-Díaz, M.L., 2012. The application of biochemical responses to assess environmental quality of tropical estuaries: field surveys. J. Environ. Monit. 14, 2608–2615.
Marques, W.S., Sial, A.N., Menor, E.A., Ferreira, V.P., Freire, G.S.S., Lima, E.A.M., Manso, V.A.V., 2008. Principal component analysis (PCA) and mineral associations of litoraneus facies of continental shelf carbonates from northeastern Brazil. Continent. Shelf Res. 28, 2709–2717.
Martín-Díaz, M.L., Blasco, J., Sales, D., DelValls, T.A., 2004. Biomarkers as tools to assess sediment quality. Laboratory and field surveys. Trac. Trends Anal. Chem. 23, 10–11.
Martín-Díaz, M.L., Gagné, F., Blaise, C., 2009. The use of biochemical responses to assess ecotoxicological effects of Pharmaceutical and Personal Care Products (PPCPs) after injection in the mussel Elliptio complanata. Environ. Toxicol. Pharmacol. 28, 237–242.
Masha'our, R.S., Heinrich, R., Garzozi, H.J., Perlman, I., 2012. Acetylcholinesterase (AChE) is an important link in the apoptotic pathway induced by hyperglycemia in Y79 retinoblastoma cell line. Front. Mol. Neurosci. 5, 69.
Mccave, I.N., Syvitski, j.p.m., 1991. Principles and methods of geological particle size analysis. In: Syvitski, J.P.M. (Ed.), Principles, Methods, and Application of Particle Size Analysis. Cambridge University Press, Cambridge, pp. 3–21.
McFarland, V.A., Inouye, S.L., Lutz, C.H., Jarvis, A.S., Clarke, J.U., McCant, D.D., 1999. Biomarkers of oxidative stress and genotoxicity in livers of field collected brown bullhead, *Ameiurus nebulosus*. Arch. Environ. Con. Tox. 37, 236–241.
Monserrat, J.M., Geracitano, L.A., Assis, H.C.S., Colares, E.P., Bianchini, A., 2006. Biomarcadores bioquímicos. In: Lana, P.C., Bianchini, A., Ribeiro, C.A.O., Nienchesk, L.F.H., Fillmann, G., Santos, C.S.G. (Eds.), Avaliação Ambiental de Estuários Brasileiros - diretrizes metodológicas. Museu Nacional/UFRJ, Rio de Janeiro, pp. 124–131.
Moraes, B.S., Loro, V.L., Glusczak, L., Pretto, A., Menezes, C., Marchezan, E., Machado, S.O., 2007. Effects of four rice herbicides on some metabolic and toxicology parameters of teleost fish (*Leporinus obtusidens*). Chemosphere 68, 1597–1601.
Moreira, L.B., Castro, I.B., Hortellani, M.A., Sasaki, S.T., Taniguchi, S., Petti, M.A.V., Fillmann, G., Sarkis, J.E.S., Bícego, M.C., Costa-Lotufo, L.V., Abessa, D.M.S., 2017. Effects of harbor activities on sediment quality in a semi-arid region in Brazil. Ecotoxicol. Environ. Saf. 135, 137–151.
Moreira, L.B., Maranho, L.A., Baena-Nogueras, R.M., Lara-Martín, P.A., Martín-Díaz, M.L., 2016. Effects of novobiocin and methotrexate on the benthic amphipod *Ampelisca brevicornis* exposed to spiked sediments. Mar. Environ. Res. 122, 169–177.
Moreira, L.B., Sasaki, S.T., Taniguchi, S., Bícego, M.C., Costa-Lotufo, L.V., Abessa, D.M.S., 2019a. Impacts of dredging on biomarkers responses of caged bivalves in a semiarid region (Ceará State, NE Brazil). Mar. Environ. Res. 151, 104784.
Moreira, L.B., Saes, R.V.S.T., Peres, T.F., Taniguchi, S., Bícego, M.C., Marins, R.V., Abessa, D.M.S., 2019b. Toxicity of sediments and dredged material from a semi-arid coastal system to marine invertebrates. Ecotoxicol. Environ. Contam. 14, 79–89.
Mottola, L.S.M., Schork, G., Resgalla Jr., C., 2009. Revisão da Metodologia e Sensiblidade do Tanaidáceo *Kalliapseudes schubartii* em Ensaios com Substâncias de Referência. J. Braz. Soc. Ecotoxicol. 4, 15–20.
Nendza, M., 2002. Inventory of marine biotest methods for the evaluation of dredged material and sediments. Chemosphere 48, 865–883.
NRC, 1997. Contaminated sediments in ports and waterways: cleanup strategies and Technologies. Committee on contaminated marine sediments, marine board. Commission on Engineering and Technical Systems. National Research Council, Washington, D.C.
Olive, P.L., 1988. DNA precipitation assay: a rapid and simple method for detecting DNA damage in mammalian cells. Environ. Mol. Mutagen. 11, 487–495.
OSPAR, 2008. Literature Review on the Impacts of Dredged Sediment Disposal at Sea. OSPAR Comission, Biodiversity Series. Publication Number 362/2008. https://www.ospar.org/documents?d=7119. (Accessed 10 January 2020).
Paula, D.P., Dias, J.M.A., Ferreira, Ó., Morais, J.O., 2013. High-rise development of the sea-front at Fortaleza (Brazil): perspectives on its valuation and consequences. Ocean Coast Manag. 77, 14–23.
Pereira, C.D.S., Abessa, D.M.S., Choueri, R.B., Almagro-Pastor, V., Cesar, A., Maranho, L.A., Martín-Díaz, M.L., Torres, R.J., Gusso-Choueri, P.K., Almeida, J.E., Cortez, F.S., Mozeto, A.A., Silbiger, H.L.N., Sousa, E.C.P.M., Valls, T.A.D., Bainy, A.C.D., 2014. Ecological relevance of Sentinels' biomarker responses: a multi-level approach. Mar. Environ. Res. 96, 118–126.
Ramos-Gómez, J., Coz, A., Viguri, J.R., Luque, Á., Martín-Díaz, M.L., DelValls, T.Á., 2011. Biomarker responsiveness in different tissues of caged *Ruditapes philippinarum* and its use within an integrated sediment quality assessment. Environ. Pollut. 159, 1914–1922.

Regoli, F., Giuliani, M.E., 2014. Oxidative pathways of chemical toxicity and oxidative stress biomarkers in marine organisms. Mar. Environ. Res. 93, 106–117.
Saes, R.V.S.T., Moreira, L.B., Davanso, M.B., Perina, F.C., Abessa, D.M.S., 2018. Developing a protocol whole sediment toxicity testing with the polychaete *Armandia agilis*. Ecotoxicol. Environ. Contam. 13, 85–97.
Saes, R.V.S.T., Moreira, L.B., Peres, T.F., Taniguchi, S., Bícego, M.C., Marins, R.V., Abessa, D.M.S., 2019. Sub-lethal responses of the polychaete *Armandia agilis* in whole sediment toxicity testing. Bull. Environ. Contam. Toxicol. 102, 310–315.
Samanta, P., Pal, S., Mukherjee, A.K., Ghosh, A.R., 2014. Biochemical effects of glyphosate based herbicide, Excel Mera 71 on enzyme activities of acetylcholinesterase (AchE), lipidperoxidation (LPO), catalase (CAT), glutathione-S-transferase (GST) and protein content on teleostean fishes. Ecotoxicol. Environ. Saf. 107, 120–125.
Sardi, A.E., Renaud, P.E., Lana, P.C., Camus, L., 2016. Baseline levels of oxidative stress biomarkers in species from a subtropical estuarine system (Paranaguá Bay, southern Brazil). Mar. Pollut. Bull. 113, 496–508.
Sardi, A.E., Renaud, P.E., Morais, G.C., Martins, C.C., Lana, P.C., Camus, L., 2017. Effects of an in situ diesel oil spill on oxidative stress in the clam *Anomalocardia flexuosa*. Environ. Pollut. 230, 891–901.
Sarkar, A., Bhagat, J., Sarker, S., 2014. Evaluation of impairment of DNA in marine gastropod, *Morula granulata* as a biomarker of marine pollution. Ecotoxicol. Environ. Saf. 106, 253–261.
Schipper, C.A., Rietjens, I.M.C.M., Burgess, R.M., Murk, A.J., 2010. Application of bioassays in toxicological hazard, risk and impact assessments of dredged sediments. Mar. Pollut. Bull. 60, 2026–2042.
Siebert, M.N., Mattos, J.J., Piazza, C.E., Lima, D., Gomes, C.H.A.M., Melo, C.M.R., Bainy, A.C.D., 2017. Characterization of ethoxyresorufin O-deethylase activity (EROD) in oyster *Crassostrea brasiliana*. Comp. Biochem. Physiol. B 203, 115–121.
Silva-Cavalcanti, J.S., Costa, M.F., 2011. Fisheries of *Anomalocardia brasiliana* in tropical estuaries. Pan Am. J. Aquat. Sci. 6, 86–99.
Silva, C.A.R., Smith, B.D., Rainbow, P.S., 2006. Comparative biomonitors of coastal trace metal contamination in tropical South America (N. Brazil). Mar. Environ. Res. 61, 439–455.
Sutter II, G.W., 2006. Ecological Risk Assessment, second ed. CRC Press, Boca Raton.
Trevizani, T.H., Figueira, R.C.L., Ribeiro, A.P., Theophilo, C.Y.S., Majer, A.P., Petti, M.A.V., Corbisier, T.N., Montone, R.C., 2016. Bioaccumulation of heavy metals in marine organisms and sediments from admiralty bay, king george island, Antarctica. Mar. Pollut. Bull. 106, 366–371.
UNEP, 1991. Determinations of Petroleum Hydrocarbons in Sediments. Reference Methods for marine Pollution Studies.
USEPA, 1991. Evaluation of Dredged Material Proposed for Ocean Disposal. Testing Manual. United States Environmental Protection Agency. EPA 503/8-91/001.
USEPA, 1992. Framework for Ecological Risk Assessment. Risk Assessment Forum. United States Environmental Protection Agency, Washington, DC. EPA/630/R-92/001.
USEPA, 1996. Method 3050B: Acid Digestion of Sediments, Sludges, and Soils. United States Environmental Protection Agency, Washington, DC.
van der Oost, R., Beyer, J., Vermeulen, N.P.E., 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: a review. Environ. Toxicol. Pharmacol. 13, 57–149.
Wills, E.D., 1987. Evaluation of lipid peroxidation in lipids and biological membranes. In: Snell, K., Mullock, B. (Eds.), Biochemical Toxicology: A Practical Approach. IRL Press, Washington, USA, pp. 127–150.
Zhang, X.J., Greenberg, D.S., 2012. Acetylcholinesterase involvement in apoptosis. Front. Mol. Neurosci. 5, 40.